

CLAIMS

What is claimed is:

1. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
  - 5 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *narGHJI*, *csn*, *yncM*,  
10 *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK* and homologues thereof; and
  - b) growing the transformed *Bacillus sp* cell of step (a) in the absence of oxygen wherein the chimeric gene of step (a) is  
15 expressed.
2. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
  - a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *narGHJI*, *csn*, *yncM*,  
20 *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK* and homologues thereof;
  - b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen whereby the cell density is increased; and  
25 c) removing oxygen from the transformed *Bacillus sp* cell of step (b) whereby the chimeric gene is expressed.
3. A method according to Claim 2 wherein after step (c) oxygen is re-supplied to the transformed *Bacillus sp* cell.  
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4. A method according to either of Claims 1 or 2 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:1-15.
5. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
  - 35 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of

- interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *feuABC*, *ykuNOP*, and *dhbABC*, and homologues thereof; and
- 5           b) growing the transformed *Bacillus sp* cell of step (a) in the absence of oxygen and in the presence of nitrite wherein the chimeric gene of step (a) is expressed.
6. A method according to Claim 5 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group
- 10           consisting of SEQ ID NOs:16-24.
7. A method according to Claim 6 wherein the concentration of nitrite is from about 1mM to about 10 mM.
8. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
- 15           a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *ycgMN*, *dhaS rapF*, *rapG*, *rapH*, *rapK*, *yqhIJ*, *yveKLMNOPQST*, *yhfRSTUV*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK*, and homologues thereof; and
- 20           b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T0 of the stationary phase wherein the chimeric gene of step (a) is expressed.
- 25           9. A method according to Claim 8 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group
- 30           consisting of SEQ ID NOs:75, 76, 25-49, and 5-15.
10. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
- 35           a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is

- selected from the group consisting of *acoABCL*, and *glvAC*, and homologues thereof; and
- 5           b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T1 of the stationary phase wherein the chimeric gene of step (a) is expressed.
11. A method according to Claim 10 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:41-44 and 50-51.
- 10           12. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
- 15           a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *yxjCDEF*, *yngEFGHI*, *yjmCDEFG*, *ykfABCD*, and *yodOPRST*; and homologues thereof; and
- 20           b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T3 of the stationary phase wherein the chimeric gene of step (a) is expressed.
- 25           13. A method according to Claim 12 the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:52-74.
14. A method according to any of Claims 1, 2 or 3 wherein the expression of the chimeric gene is down-regulated at T0 of the stationary phase.
- 30           15. A method according to any one of Claims 1, 2, 3, 4, 8, 10 and 12 wherein the *Bacillus sp.* cell is selected from the species consisting of *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus intermedius*, *Bacillus thermoamylolyticus*, *Bacillus amylolyticus*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus sphaericus*, *Bacillus stearothermophilus*, *Bacillus laterosporus*, *Bacillus acidocaldarius*, *Bacillus pumilus*, and *Bacillus pseudofirmus*.
- 35           16. The method according to any one of Claims 1, 2, 3, 4, 8, 10 and 12, wherein the coding region of interest is selected from the group consisting of *crtE*

*crtB*, *pds*, *crtD*, *crtL*, *crtZ*, *crtX crtO*, *phaC*, *phaE*, *epe*, *pdc*, *adh*, genes encoding limonene synthase, pinene synthase, bornyl synthase, phellandrene synthase, cineole synthase, sabinene synthase, and taxadiene synthase.

17. A method for monitoring the state of the cell metabolism of a *Bacillus* sp. culture comprising:

- 5 a) providing a culture of actively growing *Bacillus* sp. cells; and
- b) measuring the expression levels of a pool of genes isolated from the *Bacillus* cells of step (a), the pool of genes comprising *narGHJI*, *feuABC*, *ykuNOP*, *dhbABC*, *ydjL*, *sunA*, *yolIJK*, *csn*,  
10 *yncM*, *yvyD*, *yvaWXY*, *yhfRSTUV*, *yveKLMNOPQST*, *dhaS*,  
*rapF*, *rapG*, *rapH*, *rapK*, *ycgMN*, *yqhIJ*, *glvAC*, *acoABCL*,  
*yxjCDEF*, *yngEFGHI*, *yjmCDEFG*, *ykfABCD*, *yodOPRST*, *alsT*,  
and *yxeKLMN*, and homologues thereof.

18. A method according to Claim 17 wherein a pool of genes isolated  
15 from the *Bacillus* cells is selected from the group consisting of SEQ ID NOs:1-81.

19. A method according to Claim 17 wherein the measuring of gene expression levels is accomplished using a format selected from the group consisting of northern blots, nuclease protection assay or primer extension assays.

20. A method according to Claim 19 wherein the measuring of gene expression levels is accomplished using a nucleic acid microarray having the genes *narGHJI*, *feuABC*, *ykuNOP*, *dhbABC*, *ydjL*, *sunA*, *yolIJK*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *yhfRSTUV*, *yveKLMNOPQST*, *dhaS*, *rapF*, *rapG*, *rapH*, *rapK*, *yqhIJ*, *glvAC*, *acoABCL*, *yxjCDEF*, *yngEFGHI*, *yjmCDEFG*, *ykfABCD*, *yodOPRST*, *alsT*, and *yxeKLMN*, and homologues thereof, contained therein.

25 21. A method according to Claim 17 wherein the *Bacillus* sp. cell is selected from the species consisting of *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus intermedius*, *Bacillus thermoamylolyticus*, *Bacillus amylolyticus*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus sphaericus*, *Bacillus stearothermophilus*, *Bacillus laterosporus*, *Bacillus acidocaldarius*, *Bacillus pumilus*, and *Bacillus pseudofirmus*.

22. A method according to Claim 17 wherein the actively growing culture is grown in the absence of oxygen and the expression of genes *narGHJI*, *ydjL*, *sunA*, *yolIJK*, *csn*, *yncM*, *yvyD*, and *yvaWXY* are up-regulated in the log phase.

35 23. A method according to Claim 17 wherein the actively growing culture is grown in the absence of oxygen and in the presence of nitrite and the expression of genes *feuABC*, *ykuNOP*, and *dhbABC* are up-regulated in the log phase.

24. A method according to either of Claims 22 or 23 wherein the expression of genes *narGHJI* is down-regulated at about T0 of the stationary phase.

25. A method according to Claim 17 wherein the actively growing culture  
5 is grown in the presence of oxygen and the expression of genes *ycgMN*, *yqhIJ*,  
*ydjL*, *sunA*, *yolIJK*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *yhfRSTUV*, *yveKLMNOPQST*,  
*dhaS*, *rapF*, *rapG*, *rapH*, *rapK*, are up-regulated at about T0 of the stationary phase.

26. A method according to Claim 17 wherein the actively growing culture  
10 is grown in the presence of oxygen and the expression of genes, *acoABCL* and  
*glvAC* are up-regulated at about T1 of the stationary phase.

27. A method according to Claim 17 wherein the actively growing culture  
is grown in the presence of oxygen and the expression of genes, *yxjCDEF*,  
*yngEFGHI* *yjmCDEFG*, *ykfABCD*, and *yodOPRST* are up-regulated at about T3 of  
15 the stationary phase.

28. A method according to Claim 17 wherein the actively growing culture  
is grown in the presence of oxygen and the expression of genes, *alsT* and  
*yxekLMN* are down-regulated at stationary phase or under nutrient-limiting  
conditions.